Recovery of *Campylobacter* from Broiler Feces During Extended Storage of Transport Cages[1]

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ABSTRACT Feces deposited in transport cages by a *Campylobacter*-positive flock can cause the spread of *Campylobacter* to subsequent flocks placed in the same cages. This experiment was designed to determine the effect of extended cage storage on the viability of *Campylobacter* in feces deposited on the cage floor during commercial transport and holding. After 4 h of feed (but not water) withdrawal, *Campylobacter*-positive broilers were caught by commercial catching crews, placed into 3 new commercial cages and transported with the rest of the flock to the holding area at a commercial processing facility. Broilers were allowed to remain in the cages for 8 h before being unloaded by facility personnel. After removal of the broilers, empty cages were held under a shed and sampled at 7 intervals for the presence of viable *Campylobacter*. Cages were sampled by removing all the feces out of a different randomly assigned compartment in each cage at 0.5, 2, 4, 6, 8, 24, and 48 h after unloading. No decrease in *Campylobacter* numbers was noted through 8 h of storage. After 24 h in both replications, *Campylobacter* was detected in 2 of 3 compartments by direct plating and detected in the third by enrichment only. After 48 h, *Campylobacter* was detected in one replication by enrichment only, and was not detected in the second replication at all. Storing soiled transport cages for 48 h between uses results in lower numbers of *Campylobacter* in feces, but may not eliminate *Campylobacter* entirely. Due to cage cost and space requirements, routine cage storage between uses would not be practical.

(*Key words*: Campylobacter, feces, transport cage)

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INTRODUCTION

Many broiler flocks are found to be positive for *Campylobacter* by bacteriological culture of droppings or gastrointestinal tract contents while still on the farm. Catching, caging, and transport can have an effect on the microflora associated with broilers. *Campylobacter* numbers recovered from both whole carcass rinse samples and gut contents are higher after catching and transport than before (Stern et al., 1995). When broilers undergo the stress associated with transport, the rate of fecal shedding of *Campylobacter* increases and holding the birds at the processing plant prior to slaughter does not lessen the rate of shedding (Whyte et al., 2001). During transport and holding of broilers, fecal matter accumulates on cage flooring and is transferred to broilers held therein thus soiling feathers and skin, and causing increased bacterial counts of the unprocessed carcass (Buhr et al., 2000). *Campylobacter* can be routinely detected in swabs of commercial broiler transport cage floor surfaces (Stern et al., 2001).

It is therefore reasonable to assume that transport cages may be a point of *Campylobacter* cross-contamination in the event that a negative flock is placed into a cage after removal of positive birds. Newell et al. (2001) detected *Campylobacter* in transport cages prior to use for hauling a *Campylobacter*-negative flock and detected the same subtype of *Campylobacter* on processed carcasses from the previously negative broilers. The contamination cited by Newell et al. (2001) could have occurred during transport or elsewhere in the processing plant, but it has been shown in controlled pilot scale experiments that previously negative broilers can acquire *Campylobacter* in the transport cage (Berrang et al., 2003).

Transport cages are washed at some processing plants between uses. Twenty-eight percent of US poultry industry respondents to a water use survey routinely wash transport cages (Northcutt and Jones, 2003). Ideally, cage washing programs would lower the bacterial loads associated with transport cages. Indeed, examples of efficacious washing procedures are available in the literature (El-Assaad et al., 1995; Ramesh et al., 2002).

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However, studies conducted in a laboratory setting do not necessarily reflect commercial conditions. Unfortunately, when applied under commercial conditions, even the most stringent cage washing program may fail to completely remove pathogenic bacteria. Removal of all the feces from a cage is difficult, time consuming, costly, and requires a great deal of water. If all the feces is not removed and the entire surface sanitized, more harm than good may be done by rewetting feces already in place. Corry et al. (2002) made observations on 2 separate commercial systems used to wash plastic transport cages. They found that the washing systems were ineffective due to residual feces on cage surfaces after washing, incorrect concentration of sanitizer, and the use of contaminated recycled water to rinse washed cages. These failures resulted in transport cages that were, on occasion, still contaminated with salmonellae after washing. Similar findings with respect to *Campylobacter* were reported by Slader et al. (2002). These authors were able to detect *Campylobacter* from commercially washed transport cages in 4 out of 5 samples. Although Slader et al. (2002) reported that application of several types of sanitizers was found to lower the numbers of *Campylobacter* recovered, complete removal of *Campylobacter* was not reliably demonstrated. Broilers transported and held under commercial conditions excrete enough feces to thoroughly soil a cage. Even the soundest cage washing program may not eliminate pathogens from all surfaces of transport cages.

Because transport cages are bulky and expensive, most broiler companies do not keep many extras on hand. Therefore, in most circumstances, cages are emptied at the processing plant and immediately placed back into service to haul other broilers. Over weekends or periods of time when the processing plant is not operating, cages may be stored empty of birds. *Campylobacter* is not generally thought to be a hardy organism. Drying and exposure to atmospheric oxygen can make growth and recovery of *Campylobacter* difficult (Jay, 2000). The objective of the current study was to test the effects of extended storage between uses of transport cages on the recovery of *Campylobacter* from feces left to dry in soiled cages.

MATERIALS AND METHODS

Broilers

Commercial broiler farms housing 4-wk-old birds were sampled for *Campylobacter* by testing one house on each farm. In each house, 6 composite samples of 6 fresh fecal droppings each were taken using culture swabs.[3] Thus, 36 droppings were sampled across the entire length of the house. All swabs were replaced into their individual sterile holders, held on ice and transported to the laboratory for analysis within 2 h. When a positive house was identified, the commercial processing schedule was set to allow use of broilers from that house for the study. Broilers were subjected to 4 h of feed withdrawal but not water withdrawal in the commercial house prior to catching.

Catching, Cages, and Transport

A commercial catching crew went to the positive house and caught the birds manually, placing them into cages for transport to the processing plant. Three new live haul transport cages were included on one of the trailers to carry birds from the positive house to the plant. The new cages were assigned letters A, B, or C and designated for use in the study. Each cage was a 3-column, 5-tier dump cage[4] with steel structure, hinged doors, and fiberglass flooring in all 15 compartments. Loaded live haul trailers were transported to a commercial processing plant and broilers were held in the holding shed under conditions normal for the commercial cooperator. Broilers were in the transport cage for a total of 8 h including transport and holding time.

Eight hours after catching, dump cages A, B, and C were placed onto the conveyor belt by plant personnel and allowed to proceed through the unloading process. The live haul trailer was weighed with the empty cages for production data. Cages A, B, and C were transferred to a 5-m trailer. A data logger[5] was placed in a central compartment of each cage on the trailer to record temperature and humidity every 15 min during storage of the cages. Cages A, B, and C were transported to a 3-sided shed and held on the 5-m trailer under a roof for the duration of the study.

Sampling

At the specified time intervals, all fecal matter was scraped out of 2 randomly selected compartments from each of the 3 experimental dump cages. Fecal matter was removed using a stainless steel blade, 7.5 × 15 cm, welded on the end of a 122-cm-long steel rod. This tool was used in a manner similar to a garden hoe to pull all the feces on the floor toward the front of the coop for collection. The tool blades were flame sterilized immediately before each use. Feces from one of the compartments in each cage was placed into a sterile Whirl-Pak bag[6] for microbiological analysis. Fecal matter from the second compartment sampled at the same time from each cage was placed into a sealable bag to be used for water activity and moisture content analysis. All samples were held on ice until analyzed within 2 h.

The first sample was taken as soon as possible following removal of the broilers from the cage, 30 min in the first replication and 45 min in the second. Other samples were collected at 2, 4, 6, 8, 24, and 48 h after removal of broilers from the cages.

[3]Becton Dickinson and Co., Sparks, MD.
[4]Bright Coop Co., Nacogdoches, TX.
[5]Cox Recorders, McAdenville, NC.
[6]Nasco, Fort Atkinson, WI.

Microbiological Analysis

Fecal samples were weighed and diluted with 3 times their weight of PBS. Dilution of drier feces collected late in the study (8, 24, and 48 h after bird removal) with just 3 times the weight of PBS resulted in a paste-like suspension that was too thick to aspirate with a pipet. Therefore, when necessary, samples were diluted with 4 times their weight of PBS. Diluted samples were massaged by hand to result in a homogeneous mixture and then vigorously shaken for 30 s.

Serial dilutions of all mixed samples were made in PBS and plated onto the surface of Campy-cefex agar (Stern et al., 1992). Campy-cefex plates were incubated at 42°C for 48 h in a microaerophilic atmosphere (5% O_2, 10% CO_2, and 85% N_2).[7] All colonies characteristic of *Campylobacter* were counted. Each colony type from each sample was confirmed as *Campylobacter* by examination of cellular morphology and motility under phase-contrast microscopy. Each colony type was further characterized as being in the species *jejuni*, *coli*, or *lari* by use of a latex agglutination test kit.[8]

Samples collected at 8, 24, and 48 h after removal of broilers were enriched to detect low numbers or injured cells of *Campylobacter*. Enrichment was carried out by placing 10 mL of the diluted fecal sample into 90 mL of *Campylobacter* enrichment broth.[9] *Campylobacter* enrichment broth was incubated for 2 h at 35°C, and then transferred to 42°C for 22 h, with both incubations conducted in a microaerophilic atmosphere. Following incubation, 0.1 mL of *Campylobacter* enrichment broth was plated onto the surface of Campy-cefex agar plates. The enriched samples were incubated, examined, and confirmed as previously described.

Water Activity and Moisture Content Measurements

Water activity of feces was measured using a water activity meter.[10] Fecal moisture content was determined by the AOAC method 950.46 (AOAC, 1997) using a forced air oven. Moisture content and water activity measurements were made in triplicate except for several cases when limited sample quantity allowed for duplicate analyses only.

Statistical Analysis

Two replications of the experiment were conducted with 3 cages per replication. All *Campylobacter* counts were transformed into $\log_{10}$ colony-forming units per gram of feces. For the purposes of calculation, samples positive by enrichment only were assigned a number of colony-forming units equivalent to one-tenth the detection limit by direct plating. Detection limit by direct plating was calculated according to the dilution factor used for the feces (1:4 or 1:5) and the amount of fecal slurry placed on the lowest dilution plate (0.1 mL on each of 2 plates). An ANOVA was conducted to determine the effect of time on numbers of *Campylobacter* per gram of feces and numbers of *Campylobacter* per compartment. Individual means were compared using Tukey's honest significant difference tests, with significance defined as $P \leq 0.01$. Linear regression analyses were conducted on moisture data (water activity and percentage moisture) of feces relative to storage time. All statistical analyses were conducted with Statistica software.[11]

RESULTS AND DISCUSSION

Statistical analysis revealed no replication effect on *Campylobacter* numbers in feces left on the floor of transport cages. Therefore, data from both replications were used to calculate overall means, which are shown in Table 1. *Campylobacter* numbers per gram of feces did not change through the first 8 h of storage time, but counts per gram were significantly lower after 24 h of storage. This difference is mainly because in each replication, the compartment tested in one cage had *Campylobacter* that could be detected only by enrichment. At 48 h of storage, all compartments sampled in replication 1 were found to be *Campylobacter*-positive by enrichment only and in replication 2, no *Campylobacter* was detected in any compartment. Despite the fact that there was great variation in the amount of feces left in the compartments, the total numbers of *Campylobacter* per compartment followed the same trend as numbers per gram of feces.

During cage storage in replication 1, ambient temperature in the cages ranged from 18.3 to 31.2°C, with a mean of 24°C. Relative humidity ranged from 32 to 90.5%, with an average of 65.1%. Temperature readings peaked in late afternoon and were lowest late at night. Humidity followed an opposite trend, peaking at night and getting lower during the hottest time of day. No rain was observed during the first replication.

Water activity of feces was measured at each sample time. In replication 1, the water activity remained steady at 0.99 for the first 4 h of cage storage and then dropped steadily to 0.79 by 48 h. The change in water activity in replication 1 is described by the regression equation: $y = -0.004x + 0.98$ (y = water activity, x = h) with an R value of 0.79 and $P < 0.01$. Percentage moisture of feces was also measured at each sample time. Feces initially had 75% moisture, which dropped steadily over 48 h to 19.7%. A linear regression of this data resulted in a line described by: $y = -1.1x + 67.7$ (y = % moisture, x = h) with an R value of 0.89 and $P < 0.01$. The decrease in water available for microbiological growth and survival

[7]BOC Gases, Chattanooga, TN.
[8]Panbio INDX, Baltimore, MD.
[9]Accumedia Manufacturers Inc., Baltimore, MD.
[10]Decagon Devices Inc., Pullman, WA.
[11]Statsoft, Tulsa, OK.

TABLE 1. Mean (n = 6) $\log_{10}$ number of *Campylobacter* colony-forming units recovered from broiler feces left to dry on the floor of separate compartments in transport dump cages

Dry time (h)	Feces[1] (cfu/g)	Compartment (g of feces)	Compartment (cfu)
0.5–0.75	$7.1^{A} \pm 0.3$[2]	43.4 ± 19.8	8.7^{A} ± 0.4
2	7.2^{A} ± 0.1	28.3 ± 12.6	8.6^{A} ± 0.3
4	7.5^{A} ± 0.3	64.6 ± 62.1	9.1^{A} ± 0.6
6	7.2^{A} ± 0.2	38.3 ± 29.6	8.7^{A} ± 0.5
8	6.9^{A} ± 0.4	24.7 ± 17.0	8.2^{A} ± 0.6
24	$4.3^{B} \pm 2.8$[3]	24.6 ± 12.2	5.6^{B} ± 2.9
48	$0.3^{C} \pm 0.4$[4]	25.3 ± 9.5	1.2^{C} ± 1.3

[1]Limit of detection by direct plating was $\log_{10}$ 1.4 cfu/g. Count of $\log_{10}$ 0.7 cfu/g assigned to samples negative by direct plating but positive by enrichment. [A,B,C]Values within columns with different superscripts are significantly different ($P \leq 0.01$) by Tukey's honest significant difference test.

[2]Values ± standard deviation.

[3]Four of 6 were positive by direct plating; 2 of 6 were positive by enrichment.

[4]Three of 6 were positive only by enrichment (replication 1); 3 of 6 samples had no detectable *Campylobacter* (replication 2).

may explain the drop in *Campylobacter* levels noted at 24 and 48 h storage.

In replication 2, the ambient temperature ranged from 20.5 to 31.3°C, with a mean of 25.2°C. Due in part to 2 midafternoon rain showers, the relative humidity was higher in the second replication, ranging from 52.5 to 95.3%, with an average of 77.8%. Initial mean water activity of the fecal samples was 0.98, which remained steady for the first 4 h of storage and then began to decline slowly resulting in a water activity measurement of 0.93 at 48 h. Water activity is described by the regression equation: $y = -0.0009x + 0.97$ (y = water activity, x = h), with an R value of 0.46 and $P < 0.01$. Although the percentage moisture levels of feces in the second replication started out much lower than in the first with a mean of 55.3%, they declined more slowly over time as described by the regression formula: $y = -0.58x + 52.3$ (y = % moisture, x = h), with an R value of 0.71 and $P < 0.01$. At 48 h storage time, feces collected from the transport cages had a mean moisture of 28.9%. It would appear that the higher ambient relative humidity encountered during the second replication allowed the feces to remain moister during cage storage. It is interesting to note, however, that despite higher percentage of moisture and water activity, more *Campylobacter* was not detected in feces during replication 2 than in replication 1.

Most bacteria require a water activity of 0.9 or greater to grow (Jay, 2000). Dry conditions at relatively warm temperatures have been shown to cause stress to *Campylobacter* (Doyle and Roman, 1982). Prolonged exposure to atmospheric oxygen is also detrimental to the growth and survival of *Campylobacter*. It is possible that moisture levels encountered in replication 2 allowed *Campylobacter* cells to remain metabolically active which can make exposure to ambient oxygen levels more damaging.

In any case, extended storage of fecally soiled transport cages does not cause an increase in the numbers of *Campylobacter* left to contaminate the next flock. *Campylobacter* survives environmental hardship better at low temperatures (Doyle and Roman, 1982). Therefore, it is likely that different temperature and humidity patterns would affect these results. Under the weather conditions described, the current data show that storing a soiled transport cage in a covered shed for 48 h will lead to lower numbers of *Campylobacter* in feces. However, such storage cannot be counted on to eliminate *Campylobacter* entirely. *Campylobacter* can survive in fecally soiled transport cages for the amount of time that cages are likely to be empty during routine use.

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